International Journal of Biomedical Science and Engineering
2017; 5(4): 35-40
http://www.sciencepublishinggroup.com/j/ijbse
doi: 10.11648/j.ijbse.20170504.11
ISSN: 2376-7227 (Print); ISSN: 2376-7235 (Online)

Antibiotic Usage Pattern in Poultry and Resistance Pattern of Human Pathogenic Bacteria Isolated from Poultry Droppings in Akure, Nigeria

Kehinde Oluyemi Ajayi*, Funmilola Oluyemi Omoya

Department of Microbiology, Federal University of Technology, Akure, Nigeria

Email address:

ooluyhemikehinde@yahoo.com (K. O. Ajayi), fomoya@yahoo.com (F. O. Omoya)

*Corresponding author

To cite this article:

Kehinde Oluyemi Ajayi, Funmilola Oluyemi Omoya. Antibiotic Usage Pattern in Poultry and Resistance Pattern of Human Pathogenic Bacteria Isolated from Poultry Droppings in Akure, Nigeria. International Journal of Biomedical Science and Engineering. Vol. 5, No. 4, 2017, pp. 35-40. doi: 10.11648/j.ijbse.20170504.11

Received: February 27, 2017; **Accepted**: March 24, 2017; **Published**: October 10, 2017

Abstract: Antibiotic resistant and foodborne pathogenic bacteria of poultry origin have become important and a threat to public health, this is as a result of the continual use of the antibiotics in poultry. This study therefore aimed to provides information on Antibiotic Usage and Resistance Pattern of Human Pathogenic Bacteria Isolated from Poultry Droppings in Akure, Ondo State, Nigeria. Isolation, identification and characterization of bacterial isolates were carried out by standard microbiological method, surveillance on antibiotic usage in poultry was carried out by administering multiple choice structured questionnaires while disc diffusion method was used for antibiotic susceptibility test. Samples of fresh poultry dropping were obtained from layers, broilers chicken in Akure, Nigeria. Bacterial pathogens isolated were mainly *Enterobacteria*, *Pseudomonas aeruginosa, Staphylococcus* spp., *Bacillus* spp. and *Micrococcus luteus*. *Escherichia coli* 77 (53.50%) is the most prevalent bacteria, most poultry farm employed more than one antibiotics, twenty-one (21) different antibiotic usage patterns was observed with Enrofloxacin, NCO, Chlortetracycline and Keproceryl being the most used antibiotics. Antibiotic resistance pattern based on the most used antibiotics in poultry revealed that resistance to tetracycline 89.36% was higher in the isolates from farms that used Chlortetracycline mostly and resistance to gentamicin 51.72%, cloxacillin 100%, and erythromycin 100% was higher in those isolates from farms that used Keproceryl mostly. Conclusively, the conventional use of antibiotics in poultry may have resulted to the antibiotic resistance pattern observed in human pathogenic bacterial isolates which can be acquired by man through the food chain.

Keywords: Human Pathogenic Bacteria, Antibiotic Resistance, Antibiotic Usage Pattern, Poultry Droppings, Most Used Antibiotics

1. Introduction

There is an increasing demand for poultry meat mainly due to its acceptance by most societies and its relatively low cholesterol content and egg products. [1, 2]. The microorganisms present in the poultry droppings could be due to the contamination of the feed and water used in feeding the poultry fowl, contamination may also be caused by the microfloral of poultry attendants. Poultry meat can be contaminated by droppings with a variety of foodborne pathogens that may cause human illness following ingestion and is due to handling of raw meat, undercooking or mishandling of the cooked product [3, 4]. The world-wide increase of foodborne pathogens is of growing concern and is designated by the World Health Organisation as an emerging public health problem.

The high population density of modern intensively managed livestock operations results in sharing of both commensal flora and pathogens, which can be conducive to rapid dissemination of infectious agents. As a result, livestock in these environments commonly require aggressive infection management strategies, which often include the use of antibiotic therapy [5]. Antibiotics are used in poultry farming as: Therapeutic Agents, Prophylactic Agents and Growth

Promoters in feed [6]. It is to be stressed that in animals the antimicrobial agents are not used only for therapy and prevention of bacterial infections but also as growth promoters. In Europe, approximately 30% of all antibiotics used in animals are growth promoters [28]. Some of the commonly used antibiotics today in poultry industries in developing countries like Nigeria are Tylosine, Neomycin, Gentamycin, Tetracyclines (Chlortetracycline, Oxytetracycline), Sulfonamides (Sulfadimethoxine, Sulfamethazine, Sulfathoxazole), Penicillin (Ampicillin), Arsenicals (Roxarsone), Enrofloxacin, Erythromycine and vaccines [7].

There is growing scientific evidence that the use of antibiotics in food animals leads to the development of resistant pathogenic bacteria that can reach humans through the food chain [8]. The concern over microbial antibiotic resistance has led to the banning of antibiotic use in feeds for poultry and livestock in various countries of Europe as well as in the U.S.A [9]. It is now generally known that the main risk factor for an increase in bacterial resistance is an increased use of antibiotics, it is similar in humans and in animals [10]. These drug resistant bacteria can move then from animals to humans through a variety of channels, to farmers, their families, or employees through direct contact with animals, to those groups, their neighbors, or others through soil and water contamination or via airborne particles and consumers via contaminated meat. Bacteria also pass resistance genes back and forth, creating another mechanism by which antibiotic resistance could be transferred to human pathogens [11, 12]. This study therefore provides information on Antibiotic Usage Pattern in poultry and Antibiotic Resistance Pattern of Human Pathogenic Bacteria Isolated from Poultry Droppings in Akure, Ondo State, Nigeria.

2. Materials and Methods

2.1. Sample Locations

All samples were collected within Akure metropolis, Akure is the largest city and capital of Ondo State, located in south-west Nigeria. Akure lies about 70°15 north of the equator and 50°15 east Meridian. The city has a population of 588,000 which is 0.305% of Nigeria population based on 2006 population census, the people are of Yoruba ethnic group and are situated in the tropic rainforest. The city is a trade center for farmers where cocoa, bananas, palm oil, yams, cassava, corn, cotton and tobacco are mostly cultivated. During this research, samples of poultry droppings were collected from nine (9) different locations (FUTA, Aba, Apatapiti, Ijoka, Oritaobele, Ado road, Ondo road, Alagbaka and Lafe) in Akure metropolis.

2.2. Sample Collection

Six hundred and eighty four (644) fresh poultry droppings were collected from forty eight (48) different poultries using the method of [3, 13]. All samples were transported to microbiology research laboratory of Federal University of Technology, Akure within one hour of collection for microbiological analyses.

2.3. Isolation of Bacteria from Poultry Droppings

Bacteriological examinations were carried out using standard methods for aerobic bacteria [14]. Sample collected in Mac Cartney bottle was gently shake and stirred with sterile glass rod until the dung mixed thoroughly, aliquot (1.0 ml) was transferred into the test tube containing 9.0 ml of sterile distilled water and diluted serially in one-tenth stepwise to 10^{-7} dilution factor and 1.0 ml each of dilution 10^{-5}, 10^{-6} and 10^{-7} was pure plated on Nutrient agar and some selective and differential media (Salmonella Shigella agar, Eosine Methyline Blue agar, MacConkey agar, Manitol salt ager and Cysteine Lactose Electrolyte Deficient agar), the plates were inverted and incubated aerobically at 37°C for 24 hours after which the plates were examined for growth.

2.4. Biochemical Characterization

Different Biochemical characterisation (Gram reaction, Spore staining, Coagulase, Motility, Oxidase, Indole production, Methyl red, Voges Proskauer, Sugar fermentation, Citrate utilization, Catalase test and test for H_2S gas production) and presumptive identification of isolates were carried out as described by [15, 16].

2.5. Surveillance on Antibiotics Usage in Poultry

A Multiple choice structured questionnaires were administered to all poultry attendant and farm manager that were on duty as at the time of sample collection. In the questionnaire, the year of experience of the poultry farmer, age of birds, antibiotic that is frequently employed and number of antibiotic that have been used were put into consideration.

2.6. Antibiotics Susceptibility Test

Antibiotic susceptibility test of all the isolates was determined by the disk diffusion method and interpreted as susceptible, intermediate and resistant as described by [17]. Gram negative bacterial pathogens were tested against the following antibiotics; Tetracycline (30µg), Ofloxacin (30µg), Gentamicin (20µg), Chloramphenicol (30µg), Augmentin (30 µg), Ceftriazone (30 µg), Nitrofuratoin (300 µg), Cotrimoxazole (25 µg), Ciprofloxacin (10 µg) and Amoxicillin (30µg) while gram positive bacterial pathogens were tested against Cotrimoxazole (25 µg), Erythromycin (10µg), Gentamicin (20µg), Augmentin (30 µg), Streptomycin (10 µg), Cloxacilin (5 µg) Tetracycline (30µg) and Chloramphenicol (30µg).

2.7. Quality Control

Typed culture *(Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923) were used as quality control for antimicrobial susceptibility testing as recommend by Clinical and Laboratory Standards Institute [17].

2.8. Statistical Analysis

Data was statistically analysed using SPSS version 20, the percentage resistance were statistically analysed using

analysis of variance (ANOVA), and tests of significance carried out by Duncan's multiple range tests at $p \leq 0.05$.

3. Results

3.1. Distribution, Occurrence, Prevalence and Comparison of Bacterial Isolates Across Different Sample Locations

The occurrence of each bacterial isolates from broilers, layers and free range chicken as well as number and percentage occurrence were presented in Table 1. Total number of one hundred and forty six (146) human pathogenic bacteria was isolated from 644 samples of poultry droppings in nine (9) different locations. The result also revealed that *Escherichia coli* 77 (53.50%) is most prevalent bacterial and was isolated from layers and broilers while the least prevalent bacterial pathogens are *Enterobacter* spp. 3 (2.05%), *Shigella* spp., 2 (1.37%) *Citrobacter* spp. 3 (2.05%), *Pseudomonas aeruginosa* 2 (1.37%), *Seratia* sp. 2 (1.37%) and *Micrococcus luteus* 3 (2.05%). Also, *Staphylococcus* spp. 15 (10.27%) were more prevalent in layers 11 (73.33%) than broilers 4 (26.67%).

Table 1. *Occurrence, Prevalence and Comparison of Bacterial Isolates from Poultry Droppings in different Locations.*

		Number (%) of samples containing:												
Sample locations		***E coli* 77 (53.50)**	***Kebsiella* spp. 8 (5.48)**	***Proteus* spp. 8 (5.48)**	***Enterobacter* spp. 3 (2.05)**	***Salmonella* spp. 16 (10.96)**	***Shigella* spp. 2 (1.37)**	***Citrobacter* spp. 3 (2.05)**	***Pseudomonas* sp. 2 (1.37)**	***Seratia* sp. 2 (1.37)**	***Staphylococcus* spp. 15 (10.27)**	***Micrococcus luteus* 3 (2.05)**	***Bacillus* spp. 7 (4.79)**	**Total**
A	Layers	6	1	-	-	2	1	-	-	-	3	-	-	13
	Broilers	3	-	-	-	-	-	-	-	-	-	2	1	6
B	Layers	4	-	-	-	-	1	1	-	-	1	-	-	7
	Broilers	5	-	-	-	1	-	-	-	-	-	-	1	7
C	Layers	2	2	-	1	-	-	-	-	-	-	-	2	7
	Broilers	6	-	2	-	-	-	-	-	-	-	-	-	8
D	Layers	4	-	1	-	-	-	-	-	-	2	-	-	7
	Broilers	4	-	-	-	2	-	-	-	-	3	-	-	9
E	Layers	6	-	-	-	2	-	-	-	-	-	-	1	9
	Broilers	3	-	-	-	2	-	-	-	-	-	-	-	5
F	Layers	4	-	1	-	1	-	-	-	-	-	-	-	6
	Broilers	3	-	-	-	-	-	1	-	2	-	-	-	6
G	Layers	6	-	1	-	1	-	-	-	-	2	-	-	10
	Broilers	3	1	-	-	2	-	-	-	-	-	-	-	6
H	Layers	4	-	2	-	-	-	-	-	-	1	-	-	7
	Broilers	6	3	1	2	1	-	1	2	-	1	1	2	20
I	Layers	5	-	-	-	-	-	-	-	-	2	-	-	7
	Broilers	3	1	-	-	2	-	-	-	-	-	-	-	6

Legend: A - FUTA, B - Aba, C - Apatapiti, D - Ijoka, E - Oritaobele, F – Ado road, G – Ondo road, H - Alagbaka, I - Lafe. Values in brackets indicate percentage of total bacteria isolated, -: Not present

3.2. History of Antibiotic Usage in the Poultry Farms in Akure

The history of antibiotic been employed in the poultry farms in Akure within the period of November, 2014 and January, 2016 was accessed with the use of questionnaire and recorded in Table 2. The result revealed that different classes of antibiotics have been employed with twenty-one (21) different patterns, the number of antibiotic that have been used ranges from 5 to 1 and five (5) farms used Chlortetracycline, Enrofloxacin, Gentamycin in combination while 4 farms employed only one antibiotic. Also, most of the farms have employed at least three types of antibiotics.

Table 2. *History of Antibiotic Usage from November 2014 to October 2015 in the Poultry farms in Akure.*

Antibiotic usage pattern	Number of poultry farms
Chlortetracycline, Enrofloxacin, Gentamycin	5
Keproceryl, Gentamycin, Ciprofloxacin	4
Streptomycin, Doxycycline, Chlortetracycline	4
NCO, Gentamycin	4
Gentamycin, Keproceryl, Doxycycline	3
Keproceryl, Enrofloxacin, Neoceryl, NCO	3
Tylosin, Furazolidone, Neoceryl, Chlortetracycline	3
Chlortetracycline, Keproceryl, Enrofloxacin, Neoceryl	3
Gentamycin, Ciprofloxacin	2
Ciprofloxacin, Furazolidone, Gentamycin, Tylosin, Neoceryl	2
NCO, Chlortetracycline	2
Penicillin, Streptomycin, Tetracycline, Enrofloxacin	2
Chlortetracycline, Neoceryl	2
Gentamycin, NCO, Enrofloxacin	2
Gentamycin, Ciprofloxacin, Chlortetracycline	1
Chlortetracycline	1
Penicillin, Streptomycin, Tetracycline	1
Keproceryl	1
NCO, Penicillin	1
NCO	1
Enrofloxacin	1
Total	48

Legend: NCO- (Neomycine, Chloramphenicol, and Oxytetracycline), Neoceryl – (Neomycin, Erythromycin, Oxytetracycline, Streptomycin and Colistin), Keproceryl - (Oxytetracycline, Erythromycin, Colistin and Streptomycin)

3.3. Most Frequently Used Antibiotic in Akure Poultries

The result revealed that the most used antibiotics in Akure are Enrofloxacin, NCO (Neomycine, Chloramphenicol, and Oxytetracycline), Chlortetracycline and Keproceryl (Oxytetracycline, Erythromycin, Colistin and Streptomycin). The frequency are in this order Enrofloxacin > NCO > Chlortetracycline > Keproceryl and is presented in Table 3.

Table 3. *Most Frequently Used Antibiotic in Akure Poultries.*

Antibiotics	Frequency (percentage)
Enrofloxacin	17 (35.42)
NCO	13 (27.08)
Chlortetracycline	11 (22.92)
Keproceryl	7 (14.58)

Legend: Keproceryl - (Oxytetracycline, Erythromycin, Colistin and Streptomycin), NCO- (Neomycine, Chloramphenicol, and Oxytetracycline)

3.4. Characterisation of Antibiotic Resistance Pattern Based on Most Used Antibiotics in Poultry

Antibiotic resistance pattern of human pathogenic bacteria was characterised based on the most used antibiotic in the poultry and presented in Figure 1. The result showed that there was no significant difference ($P \leq 0.05$) in the resistance pattern shown by bacterial isolates from poultry farms that mostly used NCO (Neomycine, Chloramphenicol, and Oxytetracycline), keproceryl (Oxytetracycline, Erythromycin, Colistin and Streptomycin) and chlortetracycline, to augmentin and amoxicillin, resistance to tetracycline 89.36% was higher in the isolates from poultry farms that mostly used chlortetracycline and it was also observed that resistance to gentamicin 51.72%, cloxacillin 100%, erythromycin 100% was higher in those isolates from poultry farms that mostly used keproceryl 90.32%.

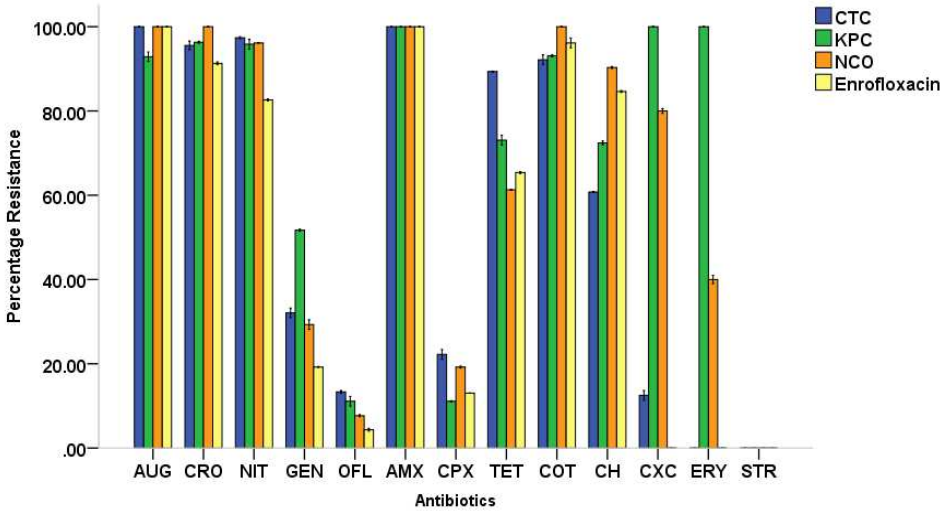

Figure 1. *Characterisation of Antibiotic Resistance Pattern based on most used Antibiotics in Poultry.*

Bars represent percentage ± standard error of resistant isolates to mentioned antibiotic, significant difference were taken at ($P \leq 0.05$) according to Duncan's New Multiple Range test

Legend: AUG- Augmentin, CRO- Ceftriaxon, NIT- Nitrofuratoin, GEN- Gentamicin, OFL- Ofloxacin, AMX- Amoxicillin, CPX- Ciprofloxacin, TET- Tetracycline, COT- Cotrimoxazole, CH- Chloramphenicol, CXC- Cloxacillin, ERY- Erythromycin, STR- Streptomycin, CTC – Chlortetracycline, KPC – keproceryl (Oxytetracycline, Erythromycin, Colistin and Streptomycin), NCO – (Neomycine, Chloramphenicol, and Oxytetracycline).

4. Discussion

In Table 1, there were variations in the distributions of these bacteria across different sample locations and also, in layers and broilers, earlier studies have shown that the growth of normal intestinal bacteria varies with the gut environment [18] which may be responsible for this. Furthermore, the probiotic and physiological state of the gut of animals has been described as one of the factors that could influence the distribution, and ultimately the recovery rate of organisms from the gut of animals [19]. Poultry meat and other poultry products can be contaminated by droppings with a variety of foodborne pathogens that may cause human illness following ingestion and is due to handling of raw meat, undercooking or mishandling of the cooked product [3, 4]. The high prevalence of *Enterobacteriaceae* in this study suggests that these families of bacteria are commensal naturally existing in the poultry gut, many countries has reported various *Enterobacteriaceae* in poultry, eggs and meats [20]. However, *Escherichia coli* has the highest prevalence in all locations and in layers and broilers, the high prevalence is in accordance with the result of [10, 21], their high prevalence could be attributed to the fact that they have a slow die off rate in the presence of selective pressure like antibiotics [22]. The presence of *Staphylococcus* species and high prevalence in layers droppings could be as a result of the continual entering of the poultry house by the workers to feed the chicken and picking of eggs.

Antibiotics are used in poultry farming as: Therapeutic Agents, Prophylactic Agents and Growth Promoters in feed [6]. The history of antibiotic been employed in the poultry farms in Akure within the period of November, 2014 and

January, 2016 was accessed Table 2 and 3, the antibiotic usage patterns revealed that all poultry farms use antibiotics to control diseases as most farms were multi-drug users and all the farms used one or more antibiotics because combination of antibiotics have been proved to be effective in poultry to combat infections, this is in agreement with previous reports [23, 24, 25, 26]. Poor environmental sanitation, un-hygienic practices, lack of biosecurity and other management inadequacies leading to increased exposure to bacterial pathogens with resultant immuno-suppression may have contributed to the observed dependence of poultry farms on antibiotics for therapeutic and/or prophylactic purposes [26]. There is growing scientific evidence that the use of antibiotics in food animals leads to the development of resistant pathogenic bacteria that can reach humans through the food chain [8]. The result obtained in this research also revealed that the most used antibiotics in Akure are Enrofloxacin, NCO (Neomycine, Chloramphenicol, and Oxytetracycline), Chlortetracycline and Keproceryl (Oxytetracycline, Erythromycin, Colistin and Streptomycin), these may be as a result of mode of actions of these antibiotics as broad spectrum and their uses have also been reported by other authors. [23], reported a high usage of quinolones, gentamycin, neomycin, tetracycline, streptomycin and tylosin among poultry farms in Ekiti State, Nigeria. Similarly [24], reported the use of enrofloxacin, tetracycline, gentamycin streptomycin, furatadone, Tylosin norfloxacin among poultry farms in Abeokuta, Nigeria, while [29] also reported the use of oxytetracycline, colistin, Tylosin and enrofloxacin among poultry farms in Khartoun, Sudan. This study also revealed that Enrofloxacin, a fluoroquinolone antibiotic is the most used antibiotic in Akure poultries which was also reported by [26], who reported Enrofloxacin as the most used drug in poultry farms in Ogun state, this antibiotic is comparatively new, expensive and are the drug of choice in the treatment of invasive enteric infections in animals. Their use may be due to their comparative higher advantage of oral preparation and potency. They also serve as a way-out for farmers that are being faced with frustrating experience with refractory bacterial infections caused by the resistant bacteria to the conventional older generation frontline antibiotics. The use of fluoroquinolones is also known to be highly successful and strategic in the treatment of salmonellosis, including infections caused by multi-resistant *Salmonella* serotypes [26, 27].

There have been concerted efforts to draw attentions to the critical problem of microbial resistance to antibiotics in recent times because the development of antibiotic resistance among pathogenic bacteria is a major public health concern, antimicrobial agents used in poultry have human analogues and increase the likelihood that bacterial pathogens of poultry origins will develop cross-resistance to antimicrobial agents used in human medicine [13]. During this research, Enrofloxacin, NCO (Neomycine, Chloramphenicol, and Oxytetracycline), keproceryl (Oxytetracycline, Erythromycin, Colistin and Streptomycin) and chlortetracycline were found to be the most used antibiotics in Akure poultries Figure 1, the result revealed that the highest resistance to gentamicin, cloxacillin and erythromycin were observed among the isolates from poultry farms where keproceryl is mostly used, the resistance may be as a result of presence of aminoglycoside and erythromycin in keproceryl, highest resistance to tetracycline, ciprofloxacin and ofloxacin were observed in isolates from poultry farms where chlortetracycline is mostly used, this may be responsible for resistance to tetracycline and may be because chlortetracycline is a tetracycline based antibiotic. Also, isolates from farms where NCO is mostly used has the highest resistance to ceftriaxone, cotrimoxazole and chloramphenicol, high resistant observed in the isolates to chloramphenicol might be as a result of the presence of chloramphenicol in NCO, isolates from farms where enrofloxacin is mostly used were also observed to be resistant to fluoroquinolone antibiotic. These observations further buttress the fact that the use of antibiotic in poultry has contributed to the emergence of antibiotic resistance.

5. Conclusion

Poultry droppings habours different bacteria, mostly enteric bacteria which are human pathogens. Among others, *Escherichia coli* is the most prevalent bacteria isolated from poultry droppings alongside with *Proteus* spp. *Klebsiella* spp. *Salmonella* spp. and *Staphylococcus* spp., it was found in all the chicken and farm locations. There is strong evidence that poultries in Akure has employed many antibiotics in their farms but Enrofloxacin, NCO (Neomycine, Chloramphenicol, and Oxytetracycline), Chlortetracycline and Keproceryl were mostly used and may have contributed to antibiotic resistant pattern of bacterial isolates. All isolated bacteria were multidrug resistant as they all showed resistance to at least three different classes of antibiotics. Since antibiotics used in poultry has greatly contributed to emergence of antibiotic resistance pathogenic bacteria in poultry and then passed to man, alternative means of therapy like the use of probiotics and phytotherapy should be research on to combat this resistance tends.

References

[1] Bolan, N. S., Szogi, A. A., Chuasavathi T., Seshadri B., Rothrock M. J. and Panneerselvam, P. (2010). Uses and management of poultry litter. *World's Poultry Science Journal*; 66.

[2] Apata, D. F. (2009). Antibiotic Resistance in Poultry. *International Journal of Poultry Science*. 8 (4): 404-408.

[3] Adegunloye, D. V., (2006). Microorganisms associated with poultry faeces. *Journal Food Agriculture and Environment.* 4: 41-42.

[4] Theodore. I. and Mbata (2007). Poultry meat pathogens and its Control. *Internet Journal of Food Safety* V (7): 20-28.

[5] Timothy, F. Landers, B. C., Thomas, E. W. and Elaine, L. L. (2012). A Review of Antibiotic Use in Food Animals: Perspective, Policy, and Potential. *Public Health Reports* (January–February, 2012): 127: 5-7.

[6] McEwen, S. A., and Fedorka-Cray, P. J. (2002). Antimicrobial use and resistance in animals. *Clinical Infectiontion Disease.* 34 Suppl 3: 93-106.

[7] Jallailudeen, R. L., Saleh, M. J., Yaqub, A. G., Amina, M. B., Yakaka, W. and Muhammad, M. (2015). Antibiotic Residues in Edible Poultry Tissues and Products in Nigeria: A Potential Public Health Hazard. *International Journal of Animal and Veterinary Advances.* 7 (3): 55-61.

[8] Van Looveren, M., Daube, G., De Zutter, L., Dumont, J. M., Lammens, C., Wijdooghe, P., Vandamme, M., Jouret, M., Cornelis, M. and Goossens, H. (2001). Antimicrobial susceptibilities of *Campylobacter* strains isolated from food animals in Belgium. *Journal of Antimicrobial and Chemotherapy*. 48: 235–240.

[9] Hayes, J. R., English, L. L., Carr, L. E. and Wagner, D. D. (2004). Multiple-antibiotic resistance of *Enterococcus* spp. isolated from commercial poultry production environments. *Appl. Environ. Microbiol*., 70: 6005-6011.

[10] Kolář, M., Pantůček, R., Bardoň, J., Vágnerová, I., Typovská, H., Válka, I. and Doškař, J. (2002). Occurrence of antibiotic-resistant bacterial strains isolated in poultry. *Veterinary Medcine Czech,* 47, (2–3): 52–59.

[11] Rushton, J., Stärk, K. and Pinto, Ferreira, J. (2014). *Antimicrobial Resistance: The Use of Antimicrobials in the Livestock Sector*. OECD Food, Agriculture and Fisheries, Papers 68. 16-21.

[12] Ward, M. J, Gibbons, C. L., McAdam, P. R., van Bunnik, B. A. D., Giravan, E. K, Edwards, G., F., Fitzgerald, J. R. and Woolhouse, M. E. J. (2014). Time scaled evolutionary analysis of the treansmission ans antibiotic resistance dynamics of *Staphylococcus aureus*. *Applied Journal of Environmental Microbiology.* 80 (23): 7275-7282.

[13] Siwela, A. H., Matsaure, F., Ncube, T., Olonitola, O. S. and Best, G. R. (2007). A comparison of antibiotic resistance in microorganisms isolated from chicken and ostrich faeces in Bulawayo, Zimbabwe. *International Journal of Biological and Chemical Science.* 1 (2): 158-164.

[14] Brown, A. E. (2005). Benson's Microbiological Applications: Laboratory Manual in General Microbiology (Reidy, P. E. and Fornango, J. S., Eds.). McGraw-Hill, Inc., New York.

[15] Fawole, M. O. and Oso, B. A. (2004). Characterization of bacteria: *Laboratory Manual of Microbiology.* 4th Edition, Spectrum Book Ltd., Ibadan, Nigeria, 24-33.

[16] Cheesbrough, M. (2006). Medical laboratory manual for tropical countries. Second edition part 1. Vol. II. Microbiology.. Cambridge University Press, London 400-480.

[17] Clinical and Laboratory Standards Institute (CLSI), (2014). Performance Standards for Antimicrobial Susceptibility Testing; *Twenty-Fourth Informational Supplement.* CLSI document M100-S24. Wayne, PA: 34 (1).

[18] Moussa, S. D., Fred, G. S., Fatoumta, D., Jane, P., Luke, M., Roland, B., Claudie, B., Pascal, D., Susan, B., Brent, J. S. and Edward, T. (2007). Impact of feed supplementation with antimicrobial agents on growth performance of broiler chicken: *Clostridium perfringens* and Enterococcus number, antibiotic resistant phenotype and distribution of antimicrobial resistance determinants in *Escherichia coli* isolates, *Applied and Environmental microbiology*, 73 (20): 6566- 6576.

[19] Ajayi, A. O. and Egbebi, A. O. (2011). Antibiotic sucseptibility of *Salmonella Typhi* and *Klebsiella Pneumoniae* from poultry and local birds in Ado-Ekiti, Ekiti-State, Nigeria. *Annals of Biological Research.* 2 (3): 431-437.

[20] Arifatun, N., Mashuk, S., Shamsun, N., Kazi, S. A., Sk, I. A, and Salequl, I. (2014). Multidrug Resistant-Proteus Mirabilis Isolated from Chicken Droppings in Commercial Poultry Farms: Bio-security Concern and Emerging Public Health Threat in Bangladesh. *Journal of Biosafety and Health Education*: 2: 120.

[21] Omojowo, F. and Omojasola, F. (2013). Antibiotic Resistance Pattern Of Bacterial Pathogens Isolated From Poultry Manure Used To Fertilize Fish Ponds In New Bussa, Nigeria. *Albanian Journal of Agriultural. Science.* 12 (1): 81-85.

[22] EPA, (2002). National Beach Guidance and Required performance Criteria for Grants, U.S Environmental Protection Agency Office of Water (4305T).

[23] Alo, O. S. and Ojo, O. (2007). Use of antibiotics in food animals; A case of a major veterinary outlet in Ekiti State, Nigeria. *Nigerian Veterinary Journal,* 28 (1): 80-82.

[24] Ogunleye, A. O., Oyekunle, M. A. and Sonibare, A. O. (2008). Multi-drug resistant *Escherichia coli* isolates of poultry origin in Abeokuta, South Western Nigeria. *Veterinarski Arhiv,* 78 (6), 501-509.

[25] Olatoye, I. O. (2011). Antibiotics use and resistance patterns of *Salmonella* species in poultry from Ibadan, Nigeria. *Tropical Veterinarian*, 29 (1): 28-35.

[26] Oluwasile, B. B., Agbaje, M., Ojo, O. E. and Dipeolu, M. A. (2014). Antibiotic usage pattern in selected poultry farms in Ogun state. *Sokoto Journal of Veterinary Sciences*, 12 (1): 45-50.

[27] Reina, J., Gomez, J., Serra, A. and Borell, N. (1993). Analysis of the antibiotic resistance detected in 2043 strains of *Salmonella enterica* subsp. *enterica* isolated in stool cultures of Spanish patients with acute diarrhoea (1986-1991*). Journal of Antimicrobial Chemotherapy,* 32 (5): 765 769.

[28] Bogaard, A. E. and Stobberingh, E. E. (2000): Epidemiology of resistance to antibiotics. Links between animals and humans. *International Journal of Antimicrobial Agents*, *14*, 327–335.

[29] Sirdar, M. M., Picard, J., Bisschop, S. and Gummow, B. (2012). A questionnaire survey of poultry layer farmers in Khartoum State, Sudan, to study their antimicrobial awareness and usage patterns', *Onderstepoort Journal of Veterinary Research* 79 (1): 8 pages.